Repeated Exposure of Infants at Complementary Feeding to a Vegetable Purée Increases Acceptance as Effectively as Flavor-Flavor Learning and More Effectively Than Flavor-Nutrient Learning1–4

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Abstract

Children’s vegetable consumption is below the public health recommendations. This study aimed to compare learning mechanisms to increase vegetable acceptance in infants at complementary feeding, namely repeated exposure (RE), flavor-flavor learning (FFL), and flavor-nutrient learning (FNL); measure the stability of the learning effect; and examine the impact of infants’ feeding history on vegetable acceptance. The study was composed of a preexposure test, an exposure period, a postexposure test, and tests at 2-wk, 3-mo, and 6-mo follow-ups. At pre- and postexposure, a basic artichoke purée and carrot purée were presented to 95 French infants (6.4 ± 0.8 mo). During the exposure period, infants were randomly split into 3 groups and were exposed 10 times to the basic (RE group; 2 kJ/g; n = 32), a sweet (FFL group; 2 kJ/g; n = 32), or an energy-dense (FNL group; 6 kJ/g; n = 31) artichoke purée 2 or 3 times/wk. To evaluate acceptance, intake (g) and liking were recorded at home by parents. Between pre- and postexposure, intake of the basic artichoke purée significantly increased in the RE (+63%) and FFL (+39%) groups but not in the FNL group; liking increased only in the RE group (+21%). After exposure, artichoke was as much consumed and as much liked as carrot only in the RE group. Learning of artichoke acceptance was stable up to 3 mo postexposure. Initial artichoke intake was significantly related to the number of vegetables offered before the study started. RE is as effective as and simpler to implement than FFL and more effective than FNL for increasing vegetable acceptance at complementary feeding. J. Nutr. 143: 1194–1200, 2013.

Introduction

Despite the well-established health protective effect of vegetable consumption, vegetable intake is consistently lower than the amount recommended by international public health guidelines (1). In particular, children eat 80% fewer vegetables than recommended (2–5). As childhood is an important period during which eating habits are acquired and because these eating habits will likely be maintained later in life (6–8), understanding how food preferences form early in life is of primary importance.

The rejection of vegetables by children could be due to their low energy density (9) and/or due to their unpleasant taste (6,10,11). Thus, it is important to study how the modification of these characteristics (energy density and taste) may affect vegetable acceptance learning in children. One key learning mechanism described in the literature is Pavlovian conditioning. It is based on an association between a neutral or disliked conditioned stimulus, such as a food, and a positive unconditioned stimulus to induce a positive shift in response to the conditioned stimulus even when the unconditioned stimulus is removed. When the unconditioned stimulus is a liked flavor, the conditioning is called flavor-flavor learning (FFL). A study on the...
effect of the FFL mechanism on vegetable acceptance, conducted with 5-y-old children, showed an increase in preference for the initially neutral vegetable when paired with a sweet taste after a conditioning period of 6 exposures (16).

Another strategy for increasing the acceptance of vegetables by infants is repeated exposure (RE). RE consists of increasing the familiarity of a food by offering it several times; it was demonstrated to be effective for increasing the acceptance of vegetables in infants at complementary feeding (17), even for an initially disliked vegetable (18).

To the best of our knowledge, the impact of FFL or FNL on vegetable acceptance has not been studied in early childhood. A comparison of the RE, FFL, and FNL mechanisms in increasing vegetable acceptance was recently conducted in older children (19,20) but has not been previously conducted at the beginning of complementary feeding. This was the aim of this investigation.

The acceptance of a new food at complementary feeding may depend on previous feeding experience. New foods would be more rapidly accepted by breast-fed infants than by formula-fed infants (17,18,21) and by infants who have been given a greater variety of vegetables since the beginning of complementary feeding (22–24). Therefore, duration of breastfeeding and variety of vegetables offered at the beginning of complementary feeding were taken into account in this study to explain acceptance of the target vegetable.

The objectives of this study were as follows: 1) to investigate and compare the efficacy of the 3 mechanisms, i.e., RE, FFL, and FNL, at increasing the acceptance (intake and liking) of a vegetable at the beginning of complementary feeding; 2) to measure the stability of the learning in the short and middle term; and 3) to examine the influence of infants’ feeding history on the acceptance of the vegetable.

Materials and Methods

The overall study design is presented in Figure 1.

Participants

Parents in the Dijon area of France were recruited using leaflets or posters distributed in health professionals’ consulting rooms, pharmacies, and day-care centers. The criteria for infant inclusion were as follows: age between 4 and 8 mo, introduction of complementary foods was started at >2 wk and <2 mo before the start of the study, no health problems or food allergies at the beginning of the study, and gestational age ≥36 wk.

This study was conducted according to the guidelines established in the Declaration of Helsinki; the study protocol was approved by the local ethics committee (Comité de Protection de Personnes Est I Bourgogne, no. 2010/32). Written and informed consent was obtained from both parents. At the end of the study, parents received a 60€ voucher.

Study foods

Food and ingredient selection. The main criteria to select the target vegetable were low consumption and neutral acceptance in young children. Because the investigation was also conducted in Denmark and England at different childhood ages, a preliminary survey was conducted to select a vegetable matching these criteria in the 3 countries (25). The target vegetable chosen was artichoke. Carrot, the control vegetable, was chosen because it was generally liked; it was supplied by the Nestlé group (NaturNes, Nestlé).

Recipe development and vegetable production. To meet European regulations for the development of food recipes to be used with children <3 y old, only baby-food-grade ingredients were used (26). The purées were produced as 100 ± 2 g jar (Freshinov). Three purée recipes were developed according to the following constraints: the basic and sweet purées had equal energy densities, but the sweet purée had a sweeter taste; the basic and energy-dense purées had comparable tastes but different energy densities. To check whether these constraints were met, sensory descriptions were conducted by a trained panel (Supplemental Table 1) and the nutritional composition was analyzed by an accredited laboratory (INZO) according to standard methods (Supplemental Table 2).

Experimental procedure

The study was designed in 6 periods: a preexposure test (pre), an exposure period, a test at postexposure (post), and tests at 2-wk (FU2W), 3-mo (FU3M), and 6-mo (FU6M) follow-ups (Fig. 1). At preexposure, postexposure, FU3M, and FU6M, one-half of the infants received a basic artichoke purée first and then a control carrot purée and inversely for the other one-half of the infants. Infants were randomly divided into 3 experimental groups, with the aim of matching groups according to age, gender, and mode of milk feeding. During the exposure period, infants were exposed 10 times to a basic (RE group), a sweet (FFL), or an energy-dense (FNL) artichoke purée. The test at 2 wk consisted in 3 measurements of the basic artichoke purée acceptance for all infants. FFL, flavor-flavor learning group; FNL, flavor-nutrient learning group; FU2W, measurement at the 2-wk follow-up; FU3M, measurement at the 3-mo follow-up; FU6M, measurement at the 6-mo follow-up; post, measurement at postexposure; pre, measurement at preexposure; RE, repeated exposure group.

FIGURE 1 Experimental design. The study was designed in 6 periods: a preexposure test, an exposure period, a postexposure test, and tests at 2 wk, 3 mo, and 6 mo follow-ups. During the tests at preexposure, postexposure, 3 mo, and 6 mo, one-half of the infants received a basic artichoke purée first and then a control carrot purée and inversely for the other one-half of the infants. Infants were randomly divided into 3 experimental groups, with the aim of matching groups according to age, gender, and mode of milk feeding. During the exposure period, infants were exposed 10 times to a basic (RE), a sweet (FFL), or an energy-dense (FNL) artichoke purée. The test at 2 wk consisted in 3 measurements of the basic artichoke purée acceptance for all infants. FFL, flavor-flavor learning group; FNL, flavor-nutrient learning group; FU2W, measurement at the 2-wk follow-up; FU3M, measurement at the 3-mo follow-up; FU6M, measurement at the 6-mo follow-up; post, measurement at postexposure; pre, measurement at preexposure; RE, repeated exposure group.
group), or an energy-dense (FNL group) artichoke purée according to their group. The FU2W consisted of 3 measurements of the basic artichoke purée acceptance for all infants. A power calculation conducted using data from a previous study (23) revealed that 24 infants by group would be necessary to observe a significant difference in intake between pre- and post-measurements. We decided to recruit at least 30 infants by group to anticipate drop-out.

All measurements were conducted at home. Parents (mostly mothers) received the study instructions from a trained experimenter, during home visits, organized before the preexposure and the 3- and 6-mo follow-ups. Phone contact was also made several times during the experimental period. Parents were instructed to choose between lunch and dinner and then to conduct the observations always at the same time. Observations had to be conducted 2 or 3 times/wk, neither on multiple occasions within the same day nor on consecutive days. They could not skip weeks, except if their infant was sick. The period ranging from pre- to postexposure lasted a mean of 41 ± 1 d. The experimental purées had to be the first food of the meal offered to infants to make sure that they were hungry. Parents were asked to warm the two 100-g jars of the same food for 20 s in a microwave or 4 min in a water bath. It was specified that during each experimental meal the parents should not give another vegetable purée. Parents were also instructed not to give other artichoke-based foods between pre- and postexposure. To check compliance, parents were asked to record the time of feeding, report which foods they offered during each experimental meal, and record each feeding occasion after the postexposure when they fed the infant an artichoke-based food.

Intake and liking measurement
For each experimental meal, intake and liking of the study foods were measured. To measure intake, parents were asked to weigh each jar before and after consumption, using a digital kitchen scale (± 1 g, Soehnle) that we provided them with, and to record the weight in a notebook. To evaluate their infant’s liking of the purée, parents were asked to use a 9-point scale ranging from 1 = dislike very much to 9 = like very much (23). They were instructed to tick the point of the scale that best reflected their infant’s liking. Parents were instructed to present the food to their infant until s/he exhibited 3 consecutive refusal signs (keeping the mouth closed, turning his/her head away, turning the spoon away, etc.).

After each observation, parents were required to rescale the jar(s) of food, freeze them, and bring the used jars back to the laboratory to check compliance with the study procedure and data accuracy.

Background information and questionnaires
Questionnaires were completed at home by parents. They reported their education level and the height (cm), weight (kg), and feeding history of their infant. They were asked if the mother had breastfed the infant or not, and if so, the duration of exclusive breastfeeding and total breastfeeding duration. They were also asked when complementary feeding had started and the foods they had offered to their infant. The number of vegetables offered before the study was calculated. These differences were analyzed by an ANCOVA, with group as factor and number of vegetables eaten before the study as a covariate. Moreover, for each group, these differences were analyzed by paired t tests between 2 times.

To test the significance of the difference of acceptance between the preexposure and the first exposure [Δ(E1 − pre); or between the 10th exposure and postexposure [Δ(post − E10)], a paired t test was calculated for each group. At pre- and postexposure, acceptance of artichoke was compared with acceptance of carrot using a paired t test.

Results
Participants
Recruitment took place between October 2010 and May 2011. During this period, 123 families expressed interest in participating in this study, but 23 of these families did not meet the inclusion criteria and were excluded. Five families dropped out during the exposure period. As a result, the data reported here are restricted to infants who completed the entire exposure period (n = 95). All of them completed the study until the FU2W; 93 completed the FU3M and 92 completed the FU6M (Supplementary Fig. 1).

The characteristics of the infants who completed the period from pre- to postexposure are presented in Table 1. The groups differed significantly only in that infants in the FFL group were weaned 2 wk later than those in the other groups. Parental education did not differ across groups.

Artichoke acceptance at preexposure and impact of infant feeding history
At preexposure (Fig. 2), no significant differences in basic artichoke purée intake were observed between the experimental groups, but liking in the FFL group was greater than in the FNL and RE groups (P = 0.026).

Among the participating infants, 19 were bottle-fed and the others were exclusively breastfed for a mean 93 ± 7 d. Duration of exclusive breastfeeding was related neither to initial intake nor to initial liking of artichoke purée.

Sixteen infants had been exposed to artichoke purée at least once before starting the study; for 15 of them, it was a commercial artichoke purée. This purée contained only 21% artichoke and the artichoke taste was hardly perceptible compared with the experimental artichoke purées. Infants had eaten a mean of 6
artichoke purée decreased significantly in the RE group between postexposure and the first exposure and between the 10th exposure and postexposure. Neither intake nor liking differed for the RE group, which always received the basic artichoke purée, between postexposure and the first exposure and between the 10th exposure and post exposure. In the FFL and FNL groups, when the artichoke purée was changed first from the basic to a sweet or energy-dense intake and liking were not significant.

Artichoke acceptance at postexposure
Between pre- and postexposure, the increase of artichoke acceptance was significant for the RE group for intake (+63%; \( P = 0.0001 \)) and liking (+21%; \( P = 0.0007 \)), for intake only in the FFL group (+39%; \( P = 0.0005 \)), and for neither intake nor liking in the FNL group (Supplemental Table 3). Consequently, at postexposure, intake and liking in the FFL and RE groups did not significantly differ from each other and were greater than in the FNL group (Fig. 2).

Intake (g)

### TABLE 1
Characteristics of infants by experimental group exposed 10 times to a basic (RE), a sweet (FFL), or an energy-dense (FNL) artichoke purée

<table>
<thead>
<tr>
<th>Group</th>
<th>Infants, n</th>
<th>Female/male, n</th>
<th>Age at preexposure, mo</th>
<th>Age at the start of complementary feeding, mo</th>
<th>BMI Z-score</th>
<th>Bottle fed, n</th>
<th>Duration of exclusive breastfeeding, d</th>
<th>Duration of total breastfeeding, d</th>
</tr>
</thead>
<tbody>
<tr>
<td>RE</td>
<td>32</td>
<td>15/17</td>
<td>6.3 ± 0.1</td>
<td>5.1 ± 0.2( ^b )</td>
<td>0.7 ± 0.2</td>
<td>6</td>
<td>79 ± 11</td>
<td>91 ± 15</td>
</tr>
<tr>
<td>FFL</td>
<td>31</td>
<td>11/20</td>
<td>6.6 ± 0.1</td>
<td>5.6 ± 0.1( ^a )</td>
<td>0.7 ± 0.2</td>
<td>7</td>
<td>78 ± 13</td>
<td>118 ± 18</td>
</tr>
<tr>
<td>FNL</td>
<td>32</td>
<td>12/20</td>
<td>6.2 ± 0.2</td>
<td>5.0 ± 0.1( ^b )</td>
<td>0.9 ± 0.3</td>
<td>6</td>
<td>60 ± 10</td>
<td>117 ± 21</td>
</tr>
</tbody>
</table>

1 Values are frequency or means ± SEMs. Means without a common letter differ, \( P < 0.05 \). FFL, flavor-flavor learning; FNL, flavor-nutrient learning; RE, repeated exposure.

**Change in artichoke acceptance during the exposure period**
The intake of artichoke purée was greater in the FFL group (141 ± 8 g, 302 ± 71 kJ) than in the RE (123 ± 7 g, 248 ± 55 kJ) and FNL (107 ± 8 g, 646 ± 50 kJ) groups. Moreover, the number of exposures affected artichoke purée intake (+4 ± 1 g/exposure; \( P = 0.0005 \)) (Supplemental Fig. 2A). Liking of artichoke purée was greater in the FFL (7.3 ± 0.2) and RE (7.0 ± 0.2) groups than in the FNL (6.1 ± 0.3) group. The number of exposures did not affect liking of the purée (Supplemental Fig. 2B). The group × exposure number interactions in the models analyzing intake or liking were not significant.

**Artichoke acceptance at follow-ups**
For each group, no significant change in basic artichoke purée intake was observed between postexposure and the different follow-ups (\( \Delta(\text{FU2W} - \text{pre}) \); \( \Delta(\text{FU3M} - \text{post}) \); \( \Delta(\text{FU6M} - \text{post}) \)) (Supplemental Table 3). Nevertheless, because the intake of basic artichoke purée decreased significantly in the RE group between the FU3M and FU6M, the group effect on intake of basic artichoke purée was no longer significant at the FU6M (Fig. 2). Compared with liking of basic artichoke purée at postexposure (Supplemental Table 3), liking at the FU3M decreased (\( \Delta(\text{FU3M} - \text{post}) \)) only in the FFL group; at the FU6M, it decreased (\( \Delta(\text{FU6M} - \text{post}) \)) for all 3 groups. Consequently to the different decreases in liking across groups, liking ratings at the FU3M did not differ in the FFL and RE groups and were greater than those in the FNL group (\( P = 0.0046 \)). No significant group effect was observed at the FU2W or the FU6M.

**Difference in artichoke acceptance between preexposure and the first exposure and between the 10th exposure and postexposure**
Neither intake nor liking differed for the RE group, which always received the basic artichoke purée, between postexposure and the first exposure and between the 10th exposure and post exposure. In the FFL and FNL groups, when the artichoke purée was changed first from the basic to a sweet or energy-dense
Carrot vs. artichoke: intake and liking

Carrot intake at preexposure was greater than artichoke intake in the RE group (115 ± 10 vs. 92 ± 9 g; P = 0.01), but not in the FNL (114 ± 10 vs. 98 ± 12 g) or FFL (122 ± 10 vs. 107 ± 8 g) groups. Infants liked carrot more than artichoke in the RE (7.1 ± 0.3 vs. 6.1 ± 0.4; P = 0.03) and in the FNL groups (7.2 ± 0.4 vs. 5.8 ± 0.5; P = 0.005) groups, but not in the FFL group (7.1 ± 0.4 for carrot vs. 7.3 ± 0.3 for artichoke).

At postexposure, carrot and artichoke intakes were not significantly different in the RE group (159 ± 13 of carrot vs. 145 ± 10 g of artichoke). Carrot intake was greater than artichoke intake in the FFL (178 ± 13 of carrot vs. 147 ± 11 g of artichoke; P = 0.007) and FNL groups (166 ± 14 g of carrot vs. 107 ± 12 g of artichoke; P = 0.0001). At postexposure, carrot and artichoke likings did not differ in the RE (7.4 ± 0.4 for carrot vs. 7.3 ± 0.3 for artichoke) or FFL (7.3 ± 0.3 for carrot vs. 7.2 ± 0.4 for artichoke) groups. Carrot liking (7.5 ± 0.3) was greater than artichoke liking (6.2 ± 0.5) in the FNL group (P = 0.01).

Discussion

The aim of this study was to investigate the effect of 3 learning mechanisms, RE, FFL, and FNL, on the acceptance (intake and liking) of a new vegetable in infants at the beginning of complementary feeding. Overall, the results show that RE and FFL were effective at increasing intake of the basic artichoke purée in the short term. Only RE appeared to increase liking. However, liking of carrot and basic artichoke were similar after exposure in the RE and FFL groups; intake of artichoke was equivalent to intake of carrot only for the RE group. On the contrary, FNL was not effective: neither intake nor liking of the basic artichoke increased in the FNL group. After the 3-mo follow-up, artichoke intake in the RE group and liking in the FFL and FNL groups decreased, leading to equivalent acceptance across the 3 groups at the 6-mo follow-up. This suggests stability of the learning effect for at least 3 mo postexposure. In fact, at the FU6M, infants were 1 y old. This age corresponds with the widening of the food repertoire (28) and the onset of food neophobia (29,30). The transition toward a more varied diet may be accompanied by a decrease in the appeal of vegetables compared with other food categories (31) and may explain the decrease in artichoke acceptance.

Thus, altogether, these results revealed for the first time that at the beginning of complementary feeding, RE and, to a lesser extent, FFL, are effective mechanisms for increasing vegetable acceptance and that the learning effect can still be observed 3 mo after the exposure period. This study lends further weight to the importance of expected satiation, which is described as the extent to which a food is expected to deliver fullness (43). In this study, infants in the FNL group learned through exposure to associate the artichoke flavor with a feeling of fullness, resulting in a lower intake compared with the other groups at the postexposure test. Moreover, the intake of the basic purée for infants of the FNL group was stable at all follow-ups (FU2W, FU3M, and FU6M), this suggests that the flavor-nutrient association formed during the exposure period was robust over time. Altogether, this result indicates that the effect of flavor-nutrient conditioning was not an increase in the affective value of artichoke but a learned satiation.

Besides, because of the fat taste of the energy-dense artichoke purée, the flavor-nutrient association could also have resulted in a flavor-flavor association. Consequently, the results suggest that FFL could be more effective with the sweet taste than with the fat taste. However, when food ingestion is considered, it is difficult to completely separate the flavor effects from the postgestive effects. Here, for ethical and regulatory reasons, it was not possible to use certain ingredients, such as sweeteners that bring sweet taste without energy or ingredients that add energy without modifying the sensory properties.

Another highlight of this study is that, at the beginning of complementary feeding, although intake and liking were lower for artichoke compared with carrot, the amount of artichoke purée eaten was high, ~100 g. This is consistent with a previous observation that infants accepted most unknown foods they were offered at complementary feeding (11). This high intake at the first exposure could explain the fact that the intake in intake over the subsequent exposures was less pronounced than in similar studies conducted with older children (19,20).

Different factors have been previously described to influence vegetable acceptance at complementary feeding, such as the milk-feeding mode (17,18,21) and the variety of vegetables eaten at the start of complementary feeding (18,22,23). In this study, duration of breastfeeding was not related to artichoke acceptance. This absence of a breastfeeding duration impact could be due to the fact that 75% of the infants had been breastfed for only a short period (~3 mo). However, the variety of vegetables eaten before the study began had a positive impact on the initial acceptance of artichoke. This result confirms that variety at the beginning of complementary feeding is particularly important for increasing acceptance of a new food (22–24).

At the 6-mo follow-up, the intake of the experimental artichoke purée did not significantly differ from the intake of the homemade artichoke purée, regardless of the group. This result supports previous results, indicating that experience with a given food can attenuate the neophobic response to other similar foods (44).

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Two types of measurement were used in this study to evaluate the infants' acceptance: intake and liking. The impact of learning differed for these 2 variables; during the exposure period, artichoke intake increased while liking was stable. Despite the fact that FFI or FN1 were supposed to induce an increase in the affective value of foods, it was not the case in this study. The present results suggest that change in intake could occur without any observable change in infants' facial or body behavior. Liking may be a less sensitive variable than intake to evaluate the effect of learning in infants. Here, liking was assessed by the parent's subjective evaluation, because they were in the best position for reporting their infant's reactions and they could compare these feeding situations with other ones.

The results of this study should be interpreted in the light of its limitations and strength. In any study conducted with infants in a natural context, at home, parents are the best intermediate, but it is difficult to completely control their practices. However, parents were given precise instructions, and data collected in the notebook revealed that they complied with the instructions.

In summary, these results highlight the plasticity of food acceptance at the moment of the transition from a milk diet to a diversified diet (21,23), revealing that conducting complementary feeding around the age of 6 mo is a favorable period for learning what and how much to eat. Moreover, this study demonstrated that repeated exposure to a basic purée is an effective and simple way to increase intake and liking not only in the short term but at least until 3 mo after exposure. This study also demonstrated that, at the beginning of complementary feeding, it is not necessary to add an ingredient with a liked taste or an energy-dense ingredient to induce learning for a novel vegetable. This is an important practical finding, as it is easier to recommend to simply repeat exposure than to add an ingredient for first food exposures. This study also confirms that at the beginning of complementary feeding, early exposure to a variety of vegetables increased acceptance for an unfamiliar vegetable.

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Literature Cited